# IN REGARD TO BACTERIAL REDUCTION OF ORGANICALLY BOUND PHOSPHORIC ACID

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# In regard to bacterial reduction of organically bound phosphoric acid.

by

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The general biological and from the judicial chemical point of view equally interesting question of the bacterial reduction of phosphoric acid is still considered controversial in view of our present knowledge.

Spieckermann[1] in the Lafarian Handbook, Kobert[2] in his Textbook of Intoxication, Kruse[3] in his General Microbology concur in referring to this question as still open. Anyone who examines the original papers will find a number of positive statements to be in crass contrast to a sequence of absolutely negative results.

The first statements about the appearance of volatile phosphor compounds during processes of putrefaction stem from Selmi[4], who verified that during slow decomposition of egg white and egg yolk, a volatile compound appears, which is not identical with hydrogen phosphide and which when dissolved in a silver solution yields a brown pre-

cipitate. In extensive and elaborate experiments conducted by Gautier and Étard[5], who performed an analysis of the gases originating during decay, the predictable presence of traces of hydrogen phosphide during putrefaction of beef and fish was noticed and these traces were found as soon as the initial decomposition gave way to a stinking putrefaction. Statements regarding the technique of verification however, are missing in this paper. Barbieri[6] as well, reported positive results when fermenting brain pulp with yeast at 38° to 45° and allegedly encountering after only a few hours considerable amounts of hydrogen phosphide. Among German authors Marpmann[7] was the first to describe the presence of volatile phosphoric compounds during decay of cheese and during the culture of various bacteria, among others the tubercle bacillus and cholera vibrio, in mediums containing phosphoric acid under anaerobic conditions. The far reaching conclusions which he developed as a result of these discoveries may very well have contributed to the fact that his statements have been disregarded even in those places where observation and technique are considered faultless. Stich[8] as well, verified the occurrence of vanishing phosphor combinations during the putrefaction at 37° of pulp made from organs accompanied by sodium alkaline reaction in the putrid gases. However he was not successful in obtaining this evidence with inoculation of various egg white bodies with bacterium coli and rotting pancrea fluid.

Stich[8] could not state from what conditions the reduction of the organically combined phosphoric acid was dependent in each singular case. Also Kreps[9] reported findings analog to Stich after experimenting with brains in an advanced rotting state. In view of the considerable importance attributed to this question particularly for forensic purposes -- the generally used procedure of Blondlot-Dusart would be measurably restricted in its application through such findings if more frequently challenged -- there was no lack of investigations into these conclusions. And the number of authors, who published negative results exclusively, is not small. As early as 1862 Fresenius and Neubauer[10] reported experiments with rotting pieces of intestines and putrid blood which were treated with Magnesium-Ammonium Phosphate. They were not successful in expelling with the aid of carbonic acid at 60° to 70° a silver phosphide forming gas. Hollefreund[11], in particular, objected to the statements of Selmi, when he obtained negative results with horse brains exposed "to the air" for four weeks and in the egg yolk which had been left "in air" at temperatures not exceeding 15°. Klett[12] who investigated the problem of reduction through bacteria and who cultivated them in cultures treated with sodium phosphite, likewise arrived at negative results. He never did observe glowing of the culture, which, as he expected, would stem from the phosphor resulting from the reduced phosphoric acid. Hallasz[13] in-

vestigated the usefulness of the Blondlot-Dusart sample but never could positively encounter it in animal organs which had been left to rot in cool locations. It even turned out to be negative in situations in which phosphor had been added. Fischer[14] too, who left his substrate, mostly potatoes, to rot for only a few days, in one experiment inoculated with bacteria coli, never obtained volatile phosphor compounds. His short-lived experiments can hardly be evaluated as decomposition experiments. Yokote[15], who occupied himself exclusively with this problem, rejected in advance all experiments in which besides tin foil, lead foil as well had been blackened. A number of seemingly positive findings were explained by him as caused by the transfer of phosphor from the rubber material into the present hydrobromic acid or to be caused by the formation of silizium dioxide - ammonia molybdate. Only one experiment was conducted by him under anaerobic conditions and this one proved to be negative. Finally, more recently, Lemkes[16] reports negative results in regard to a re-examination of the Blondlot-Dusart procedure by means of experiments conducted on human brains in various stages of decomposition.

These contradictions and the concern in regard to the Blondlot-Dusart procedure, originating therein motivated Ehrenfeld and Kulka[17] to modify it, whereby only the phosphoric resp. subphosphoric acid which was formed could be proven to exist. In the course of these investigations, ex-

periments at decomposition of rabbit organs were tried. These organs were soaked in sodium phosphate, buried superficially and left to rot for several months during the cold season. Investigated subsequently by Mitscherlich and Ehrenfeld-Kulka, the conclusions proved to be negative. On the other hand, Kulka[18] was able to demonstrate without any doubt the presence of vanishing phosphor conbinations in the gases bubbling up from the decay room of a sewage treatment plant. In about 6 liters of gas phosphor corresponding to 2.1 magnesium pyrophosphate was found. Anaerobically conducted experiments, in which bacillus putrificus was cultivated in a pure culture at 37° in brains and egg yolk, yielded unquestionably rapidly disappearing phosphor compounds in the putrid gases.

A survey of these statements found in the literature yields numerous contradictions in both the positive and the negative statements. In part the questioning is completely misdirected; but almost universally the objection can be raised, disregarding for the moment any objections against the methods used in obtaining the evidence, that the conditions of the decomposition in the various, each other contradicting, papers were entirely different so that no pro or con can be derived from them.

Our immediate motivation in resuming this subject can be found in a court ordered chemical examination which one of us jointly with H. Jansch of the medical-chemical institute performed. It concerns a sudden death whereby the

suspicion of a murder by poison could not be ruled out. Neither the history of the ailment nor the autopsy records offered even the faintest starting point to assume phosphoric poisoning. The examination according to Mitscherlich also turned out to be negative. The test according to Blondlot-Dusart, however, yielded clearly positive results. In the further course of the investigation the case was solved as arsenic poisoning with unusually large amounts of the poison. The positive result of the Blondlot-Dusart test gave rise to the idea, that we were dealing here with a bacterial reduction of organically bound phosphoric acid. In fact the parts of the corpse had been lying for almost two weeks in the post office during the most severe summer heat because of a false mailing address and were in a state of extreme decomposition when finally handed over. The blood of the cadaver in particular which had been mailed in a bottle with fitted stop and was additionally wrapped in parchment was in an extreme state of decomposition and at opening of the bottle released under pressure a gas with a strong odor of hydrogen sulphide. The proof that in the case under consideration the result of the Blondlot-Dusart test could actually be affected by bacterial reduction was supplied by experiments which we performed on the blood of cattle.

Fresh blood from cattle was obtained at the slaughter house and though not germ free was filled into sterile flasks of 200 ccm. capacity in portions of 150 ccm. and

each flask inoculated with five drops of the blood drawn from the corpse. Two of the flasks were covered with layers of paraffin and one pair of paraffin covered flask and one pair of uncovered flask kept in an incubator at 37° and 22° respectively. Alongside controls of non-inoculated cattle blood were placed under identical conditions. Soon an intensive gas formation developed in the anaerobically maintained inoculated flasks, however we refrained from examining the gases. At the end of 38 days at first fifty ccm. of the anaerobically at 37° kept blood was worked on directly per Blondlot-Dusart. The zinc and the sulphuric acid used for the formation of hydrogen had been tested and found to be perfect. The apparatus was placed in a dark room. Soon an appreciable dark brown precipitate formed in the silver-nitrate receiver which was further processed in typical fashion after developing for two more weeks. Should by chance hydrogen sulphide form, it would be captured by an attached tube by means of solid potassium and sodium hydroxide lime. There became clearly visible an emerald green coloration of the cone of the hydorgen flame which took on an even more beautiful appearance when inserting a porcelain dish. The escaping gas evidenced the typical leekish odor of hydrogen phosphide. Just like the anaerobic sample kept at 37°, the anaerobic sample kept at 22° also evidenced a positive though weaker precipitate. The

aerobically maintained samples and the controls, processed in the same manner had completely negative results.

Therewith it was shown irreproachably that bacteria under anaerobic conditions at suitable temperature are capable of reducing organically bound phosphoric acid sufficiently so as to obtain a positive Glondlot-Dusart reaction. The manifold challenged positive statements of the older literature were finding confirmation through our results. At the same time however the door was opened to deal with the entire problem in a systematic manner.

To further clarify the conditions of this reduction we next set about to cultivate the bacteria found to be in the putrid mixture used. By means of aerobic cultivation two strains were isolated, one of which was determined to be strain L known as Proteus Zenckeri, the second strain M, which liquified gelatine, can be considered to belong to the Proteus group, although it deviates in a few instances from the typical bacterium vulgare. A germ count led to the conclusion that under aerobic conditions it only grew three times as densely in blood as under exclusion of oxygen. Both strains, under conditions identical to those above, were subjected to experiments performed on human or canine blood obtained by Venae punctio under sterile precautions. Its result was completely negative. We can therefore state for the time being that the optional anaerobiants examined by us are by themselves not capable for the reduction of

phosphoric acid. We began experiments on the indispensable anaerobiants from our cultures, as well as with others with known reduction capacity. The question of symbiotic cooperation, which Kämmerer[19] established during the reduction of haematin to haematoporphyrin by intestinal bacteria, shall receive appropriate attention. In view of the practical implications, however, we feel ourselves obligated to publish immediately the available result of our effort.

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5.) Gautier and Étard, C. r. 94, 1357, 1882.

6.) Barbieri, C. r. 131, 347, 1900.

7.) Marpmann, Zentralbl. f. Bakter. 1, 22, 582, 1897.

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15.) Yokote, Arch. f. Hyg. 50, 118, 1904.

16.) Lemkes, ref. Chem. Zentralbl. 1917, 1, 604. Nothing could be ascertained from the reference about the technique of the decay experiments.

17.) Ehrenfeld and Kulka, Hoppe-Seyler 59, 53; 63, 315.

18.) Kulka, Zentralbl. f. Bakter., Abt. 1, 61, 336, 1912.

19.) Kämmerer, 35. Kongress f, inn. Med. Wien 1923.